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Short communication

Dopamine D4 receptor (D4R) deletion in mice does not affect operant responding for food or cocaine

Panayotis K. Thanos[a,b,c,d,*], Rahila Habibi[b,c], Michael Michaelides[b,c], Ujval B. Patel[b], Katherine Suchland[e], Brenda J. Anderson[c], John K. Robinson[c], Gene-Jack Wang[b], David K. Grandy[e], Nora. D. Volkow[a,b]

[a] Laboratory of Neuroimaging, NIAAA, NIH, Bethesda, MD, United States
[b] Behavioral Neuropharmacology and Neuroimaging Lab, Department of Medicine, Brookhaven National Laboratory, Upton, NY, United States
[c] Department of Psychology, State University of New York at Stony Brook, Stony Brook, NY, United States
[d] Neuroscience, State University of New York at Stony Brook, Stony Brook, NY, United States
[e] Physiology and Pharmacology, Oregon Health Sci. University, Portland, OR, United States

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ABSTRACT

In this study we examined the genetic contribution of the D4R in food and cocaine self-administration using D4R mice. Mice were examined for operant responding to food pellets or intravenous cocaine. Compared to wild-type mice ($D4R^{+/+}$), both heterozygous ($D4R^{+/-}$) and knockout ($D4R^{-/-}$) mice showed no difference in responding for food or cocaine. Our findings suggest that the D4R is not directly involved in mediating operant response behaviors for food or cocaine.

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1. Introduction

The dopamine D4 receptor (D4R) is predominantly located in cortical regions in both pyramidal and GABAergic cells [1] and in striatal neurons [2]. It has been proposed that D4R act as an inhibitory postsynaptic receptor controlling the neurons of the frontal cortex and striatum [3]. High density of D4Rs also occurs in hippocampus and thalamus [1]. The gene encoding for the D4R is highly polymorphic and variations in the VNTR region of the third loop have been associated with substance abuse and addiction [4], drug craving [5], obesity [6], binge eating [7], ADHD [8] and novelty seeking [9]. Recent studies have shown that the 7-repeat allele of the D4R is associated with prefrontal cortex dependent working memory function [10] and in cortical thinning in regions that play a major role in attention [11].

Preclinical studies have also implicated the D4R in processes that regulate food intake such as satiety [12] and cue-induced food craving [13]. Similarly, recent research suggested distinct mechanisms for D4R modulation of the reinforcing (perhaps via attenuating dopaminergic signaling) and locomotor properties of different psychostimulant drugs [14]. Furthermore, previous findings suggest that individuals with D4R polymorphisms might show enhanced reinforcing responses to methylphenidate and amphetamine and attenuated locomotor response to amphetamine [14] and novelty seeking [15], which is a characteristic associated with the propensity for drug self-administration.

However little is known about the involvement of D4R directly on feeding and/or drug reward processes. To address these questions, we assessed the effects of partial or complete D4R deletion in mice on operant responding for food and cocaine.

2. Methods and materials

2.1. Animals

All mice (originally obtained from D.K. Grandy) were bred at Brookhaven National Laboratory and generated as previously described [16]. A total of 27 male adult mice (6–7 months old) were used for the food SA experiment [$D4R^{+/+}$ ($N=6$), $D4R^{+/-}$ ($N=11$), $D4R^{-/-}$ ($N=10$)]. A total of 26 mice were used in the cocaine SA experiment [$D4R^{+/+}$ ($N=8$), $D4R^{+/-}$ ($N=11$), $D4R^{-/-}$ ($N=7$)]. All mice were individu-

Abbreviations: DA, dopamine; D4R, dopamine D4 receptor; D2R, dopamine D2 receptor; SA, self-administration; IV, intravenous.

* Corresponding author at: Behavioral Neuropharmacology and Neuroimaging Lab, Department of Medicine, Brookhaven National Laboratory, Upton, NY, United States. Tel.: +1 631 344 7364; fax: +1 631 344 5311.
E-mail address: thanos@bnl.gov (P.K. Thanos).

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ally housed and maintained in a controlled room (68–72°F and 40–60% humidity) with a 12-h reverse light–dark cycle (lights off at 08:00 h). All mice were food-restricted to maintain an 85% body weight of age-matched ad libitum fed mice during operant training with food. The mice used in the food SA experiment continued on this regimen while those used in for cocaine SA were switched to an ad libitum regimen after operant training. Experiments were conducted during the dark cycle from 10:00 to 13:00 h. All experiments were conducted in conformity with the National Academy of Sciences Guide for Care and Use of Laboratory Animals [17] and Brookhaven National Laboratory Institutional Animal Care and Use Committee protocols.

2.2. Food self-administration

Operant chambers (30 cm × 25 cm × 30 cm; Coulbourn Instruments, Allentown, PA) were placed inside sound attenuation cubicles. Each operant chamber contained 2 levers and a food receptacle in between. Animals were tested on a fixed-ratio 1 (FR1) schedule and each session lasted 90 min. The left lever was designated as the "active" lever and responses to it led to the delivery of a 20 mg food pellet (Research Diets: New Brunswick, NJ), while the right lever was designated as the "inactive" lever and responses to this did not have any consequences but were recorded. Both levers were situated directly under their respective cue lights and pellet delivery was signaled by the onset of the cue light above the lever. After the food pellet delivery, the cue light turned off and there was a time-out for 30 s where no food was available. SA experimental variables were programmed using Graphic State v3.02 software (Coulbourn Instruments, Allentown, PA). Mice were first trained for food reinforced lever responding until responding met baseline criteria adopted from previous SA studies [18,19]. After this training phase mice were tested for food SA for a period of 10 days. Behavioral variables assessed consisted of the number of active and inactive lever presses and number of food pellets delivered.

2.3. Cocaine self-administration

After the same food training schedule as in the food SA experiment, mice received indwelling catheters in the right jugular vein as previously described [20]. Mice were then given a 3-day recovery period following jugular catheter surgery. After the recovery period mice were tested on cocaine SA for a period of 10 days. Cocaine SA experiments followed the same protocol and design as food SA experiments with the exception that instead of a food pellet, mice were injected with 1 mg/kg of cocaine delivered at a fixed rate of 0.025 ml/s for duration of 2 s. Catheter patency was tested using a 50/5 mg/kg ketamine/xylazine solution every 3 days and at the end of the 10-day period. Catheters were deemed patent only if mice lost the righting reflex within 3 s of the injection. Mice that failed the patency test at any time point were excluded from the study.

3. Results

3.1. Food self-administration

3.1.1. Lever responses

For active lever responses a two-way ANOVA showed no significant (D4R) genotype effect ($F(2, 230) = 0.82$; p = ns; Fig. 1). We did find a significant main effect for Time ($F(9, 230) = 4.68$; $p < .001$; Fig. 1) but no interaction effects ($F(18, 230) = 0.64$; p = ns). For inactive lever responses a two-way ANOVA showed no significant main effects for Strain ($F(2, 231) = 3.99$; p = ns) and Time ($F(9, 231) = 2.82$; p = ns) as well as their interaction ($F(18, 231) = 1.56$; p = ns).

3.2. Number of pellets

For pellets delivered, a two-way ANOVA showed no significant (D4R) genotype effect ($F(2, 226) = 1.03$; p = ns; Fig. 2). We did find a significant main effect for Time ($F(9, 226) = 6.49$; $p < .001$) but no interaction effects ($F(18, 226) = 0.83$; p = ns).

3.3. Cocaine self-administration

3.3.1. Lever responses

For active lever responses a two-way ANOVA showed no significant (D4R) genotype effect ($F(2, 224) = 1.41$; p = ns; Fig. 1). We did find a significant main effect for Time ($F(9, 224) = 7.12$; $p < .001$) but no interaction effects ($F(18, 224) = 0.86$; p = ns). For inactive lever responses a two-way ANOVA showed no significant main effects for Strain ($F(2, 224) = 1.17$; p = ns) and Time ($F(9, 224) = 2.19$; p = ns) as well as their interaction ($F(18, 224) = 0.62$; p = ns).

3.4. Number of cocaine infusions delivered

For cocaine infusions, a two-way ANOVA showed no significant (D4R) genotype effect ($F(2, 215) = 1.00$; p = ns; Fig. 2). We did find a significant main effect for Time ($F(9, 2150) = 6.41$; $p < .001$) but no interaction effects ($F(18, 215) = 0.55$; p = ns).

4. Discussion

Here we show that the D4R is not involved in modulating the operant reinforcing effects of both natural (food) and drug rewards (cocaine). Our results indicate that mice with lower D4R levels ($D4R^{+/-}$) or totally lacking D4R ($D4R^{-/-}$) showed similar behavioral responses as wild-type mice, which suggests that the D4R has limited involvement in food and cocaine consumption behavior.

$D4R^{-/-}$ mice have been shown to have lower dopamine release in reward-related mesolimbic areas of the brain [21]. Based on this finding, it would seem that $D4R^{-/-}$ mice would be more vulnerable to the rewarding effects of food and cocaine. Indeed studies have shown that $D4R^{-/-}$ mice are more sensitive to the motor effects

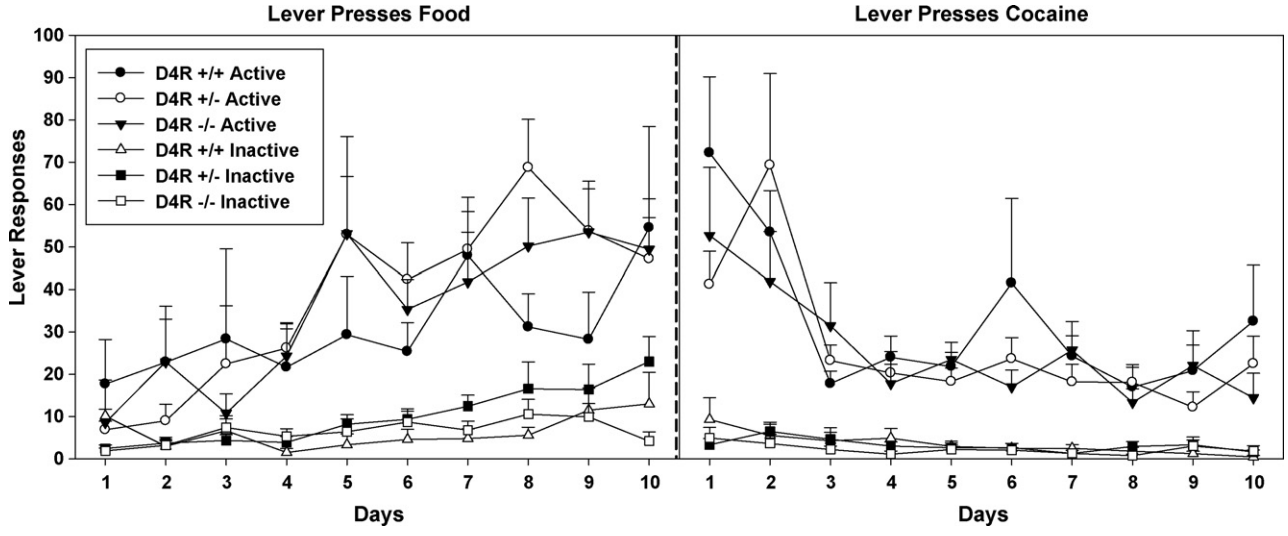

Fig. 1. Mean lever responses during food and cocaine self-administration.

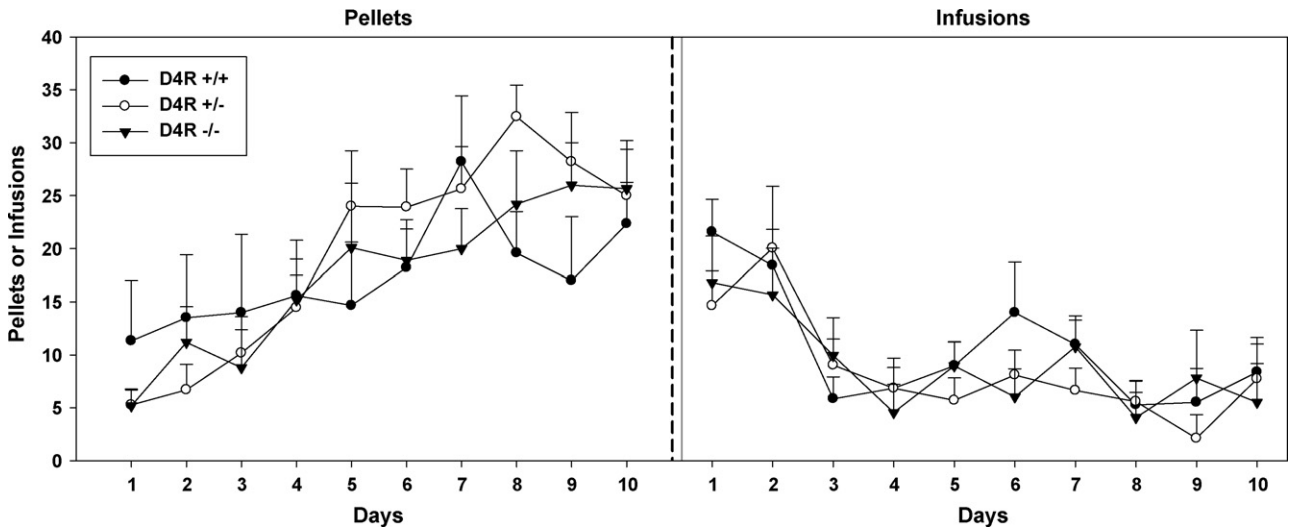

Fig. 2. Mean pellets and infusions delivered during food and cocaine self-administration.

of ethanol, cocaine, methamphetamine [16,22] and amphetamine [23] compared to wild-type mice. However, aside from this motor hypersensitivity to ethanol, the two genotypes do not differ in alcohol consumption [24]. Similarly, even though cocaine induces greater motor effects in D4R$^{-/-}$ than in wild-type mice, we previously found no difference in conditioned place preference for cocaine between D4R$^{-/-}$ and D4R$^{+/+}$ mice [14]. One study reported that D4R$^{-/-}$ mice were more sensitive to the discriminative-stimulus effects of 10 mg/kg IP cocaine than wild-type mice [22]. However, this study concluded that these effects were probably not mediated by the D4R (and probably due to changes in D2R and D3R due to D4R deletion) since the D2R/D3R antagonist raclopride (which has negligible affinity for D4R) produced similar changes in discriminative-stimulus behavior to cocaine in both strains [22]. In addition, the highly selective D4R antagonist L-745,870 did not affect discriminative-stimulus effects of cocaine in rats [19,25]. Finally, a recent study showed that D4R$^{-/-}$ mice do not differ from wild-type mice in measures of impulsivity [26]. This finding substantiates our results since impulsive behavior is linked to the propensity to seek and consume food [27] as well as cocaine [28,29].

When taken together with these prior findings, our results suggest that the previously observed hypersensitivity of D4R$^{-/-}$ mice to drugs and alcohol may be specific to motor behavior and may not translate to other behaviors specific to food and drug seeking or consumption. This view however may be limited in part by the non-specificity of the genetic manipulation associated with transgenic models. The D4R has been linked to novelty seeking [30] and has therefore been suggested play a role in substance abuse disorders. In particular, D4R polymorphisms in humans have been linked to alcohol [4] and opiate abuse [31]. Our findings suggest that such predispositions may be due to indirect effects of D4R polymorphisms on other brain mechanisms. Future studies are aimed at evaluating the profile of D2R and D3R in D4R$^{-/-}$ mice will prove useful in validating this view.

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